# Distribution of virulence factors in ESBL-producing Escherichia coli isolated from the environment, livestock, food and humans

Müller, Andrea ; Stephan, Roger ; Nüesch-Inderbinen, Magdalena

Abstract: In this study, extended-spectrum ß-lactamase (ESBL)-producing Escherichia coli isolates recovered from the following sources were characterized with regard to the occurrence and distribution of uropathogenic and enteric pathogenic virulence factors: surface waters (rivers and lakes, n=60), the intestines of freshwater fish (n=33), fresh vegetables (n=26), retail poultry meat (n=13) and the fecal samples of livestock (n=28), healthy humans (n=34) and primary care patients (n=13). Among the 207 isolates, 82% tested positive by PCR for one or more of the virulence factors (VF) that predict uropathogenicity, TraT, fyuA, chuA, PAI, yfcv or vat. Uropathogenic E. coli (UPEC) were detected in each of the analyzed sources. Regarding virulence factors for intestinal pathogenic E. coli, these were found more rarely and predominantly associated with the aquatic environment, with aagR (EAEC) found in isolates from surface waters and STp (porcine heat stable enterotoxin) and LT (heat-labile enterotoxin) associated with isolates from fish. Aggregate VF scores (the number of unique virulence factors detected for each isolate) were lowest among isolates belonging to phylogenetic group B1 and highest among group B2. Clustering of the isolates by phylogenetic group, multilocus sequence type (MLST) and ESBL-types revealed clonal overlaps of A:ST10(CTX-M-1) and D:ST350(CTX-M-1) between the sources of livestock, poultry meat and healthy humans, suggesting livestock, in particular poultry, represents a potential reservoir for these particular UPEC clones. The clones A:ST10(CTX-M-55) and B2:ST131(CTX-M-27), harboring uropathogenic virulence factors were significantly associated with fresh vegetables and with fish, respectively. Further clonal complexes with source overlaps included D:ST38(CTX-M-14), D:ST69(CTX-M-15), D:ST405(CTX-M-15) and D:ST648(CTX-M-15), which were found in surface water and healthy humans. Identifying potential reservoirs of UPEC in the environment, animals, food and humans is important in order to assess routes of transmission and risk factors for acquiring UPEC

DOI: https://doi.org/10.1016/j.scitotenv.2015.09.135

Müller, Andrea; Stephan, Roger; Nüesch-Inderbinen, Magdalena (2016). Distribution of virulence factors in ESBL-producing Escherichia coli isolated from the environment, livestock, food and humans. Science of the Total Environment, 541:667-672.
DOI: https://doi.org/10.1016/j.scitotenv.2015.09.135

# Distribution of virulence factors in ESBL-producing *Escherichia coli* isolated from the environment, livestock, food and humans

Andrea Müller[1]

[1]Institute for Food Safety and Hygiene, University of Zurich, Winterthurerstr. 272, 8057 Zurich, Switzerland.

andrea.mueller3@uzh.ch

Roger Stephan[1]*

[1]Institute for Food Safety and Hygiene, University of Zurich, Winterthurerstr. 272, 8057 Zurich, Switzerland.

stephanr@fsafety.uzh.ch

Magdalena Nüesch-Inderbinen[1]

[1]Institute for Food Safety and Hygiene, University of Zurich, Winterthurerstr. 272, 8057 Zurich, Switzerland.

magdalena.nueesch-inderbinen@uzh.ch

*Corresponding author

Roger Stephan: stephanr@fsafety.uzh.ch

**Abstract**

In this study, extended-spectrum ß-lactamase (ESBL)-producing *E. coli* isolates recovered from the following sources were characterized with regard to the occurrence and distribution of uropathogenic and enteric pathogenic virulence factors: surface waters (rivers and lakes, n=60), the intestines of freshwater fish (n= 33), fresh vegetables (n=26 ), retail poultry meat (n= 13) and the fecal samples of livestock (n= 28), healthy humans (n= 34) and primary care patients (n=13). Among the 207 isolates, 82% tested positive by PCR for one or more of the virulence factors (VF) that predict uropathogenicity, *TraT*, *fyuA*, *chuA*, PAI, *yfcv* or *vat*. Uropathogenic *Escherichia coli* (UPEC) were detected in each of the analyzed sources. Regarding virulence factors for intestinal pathogenic *E. coli*, these were found more rarely and predominantly associated with the aquatic environment, with *aagR* (EAEC) found in isolates from surface waters and STp (porcine heat stable enterotoxin) and LT (heat-labile enterotoxin) associated with isolates from fish. Aggregate VF scores (the number of unique virulence factors detected for each isolate) were lowest among isolates belonging to phylogenetic group B1 and highest among group B2. Clustering of the isolates by phylogenetic group, multilocus sequence type (MLST) and ESBL-types revealed clonal overlaps of A:ST10(CTX-M-1) and D:ST350(CTX-M-1) between the sources of livestock, poultry meat and healthy humans, suggesting livestock, in particular poultry, represents a potential reservoir for these particular UPEC clones. The clones A:ST10(CTX-M-55) and B2:ST131(CTX-M-27), harbouring uropathogenic virulence factors were significantly associated with fresh vegetables and with fish, respectively. Further clonal complexes with source overlaps included D:ST38(CTX-M-14), D:ST69(CTX-M-15), D:ST405(CTX-M-15) and D:ST648(CTX-M-15), which were found in surface water and healthy humans. Identifying potential reservoirs of UPEC in the environment, animals, food and humans is important in order to assess routes of transmission and risk factors for acquiring UPEC.

**Keywords**

Uropathogenicity, Enterobacteriaceae, CTX-M, clones, environmental sources.

## 1. Introduction

*Escherichia coli* is a bacterial species of multitudinous characteristics that occurs naturally in the digestive tract of humans and warm-blooded animals. Apart from non-pathogenic commensal isolates, two subdivisions of *E. coli* are, by virtue of their acquisition of virulence factors (VF), etiological agents of intestinal or extraintestinal diseases.

One first major group of pathogenic *E. coli* causes characteristic symptoms of gastrointestinal disease and consists of the pathotypes enteropathogenic *E. coli* (EPEC), shiga toxin-producing *E. coli* (STEC) and its subgroup enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusively adhesive *E. coli* (DAEC). (Nataro & Kaper, 1998). A second major group of pathogenic *E. coli* cause infections outside the gastrointestinal system and are termed extraintestinal pathogenic *E. coli* (ExPEC). This group includes avian pathogenic *E. coli* (APEC), which causes respiratory tract infections and septicaemia in poultry and uropathogenic *E. coli* (UPEC) (Kaper et al., 2004). Principally, the human intestinal tract is thought to be the primary reservoir for UPEC from where it can disseminate to the urogenital tract, causing in an ascending manner, urinary tract infections (UTIs) (Pitout 2012; Singer 2015).

Virulence factors are distributed unequally among commensal and pathogenic *E. coli*, enabling a classification according to phylogenetic group (Clermont et al., 2000). Thereby, most commensal stains belong to phylogenetic group A or B1, and extraintestinal pathogenic strains, which possess more VF than commensal strains, are assigned to phylogenetic groups B2 or D. Whereas for enteropathogenic *E. coli* each pathotype can be characterized and related to disease symptoms by its specific combination of VFs, (Kaper et al., 2004), there exists to date no concrete set of virulence factors for defining an *E. coli* as ExPEC or for distinguishing ExPEC subgroups from one another (Singer 2015). Although a basic virulence

gene profile exists for both UPEC and APEC, VFs that are specific to UPEC and that can clearly distinguish it from APEC have not yet been identified (Wiles et al., 2008). Some studies therefore state that some pathogenic as well as non-pathogenic strains in domestic bird populations represent potential UPEC strains in humans (Danzeisen et al., 2013; Johnson et al., 2003; Maluta et al., 2014).

UTIs are among the most frequent human bacterial infections, and constitute a major global burden of disease (Marrs et al., 2005; Totsika et al., 2012). Consequently, the emergence during the last two decades of UPEC harboring antimicrobial resistance genes is a particular threat to human health (Pitout 2012). Many multidrug resistant *E. coli* strains that are commonly isolated from UTIs belong to specific worldwide endemic clones and have been detected in surface waters and water-related environments (Amos et al., 2014; Tausova et al., 2012; Zurfluh et al., 2013). These clones include the multidrug resistant, extended-spectrum ß-lactamase (ESBL)-producing *E. coli* B2:ST131 or the trimethoprim-sulfamethoxazole resistant *E. coli* Clonal Group A (CGA), a clone that clusters within phylogroup D: ST69 (Totsika et al., 2012). Identifying further potential reservoirs of these and other virulent ExPEC clones may help understand the way they spread throughout the environment.

The purpose of this study was to determine the occurrence and distribution of virulence genes in a collection of ESBL-producing *E. coli* isolates originating from a broad range of environmental, food, animal and human sources. These sources included rivers, lakes, freshwater fish, vegetables, livestock, retail chicken meat, healthy humans and primary care patients.

## 2. Materials and Methods

### 2.1. Strain collection

The collection of ESBL-producing strains consisted of 60 isolates from rivers and lakes in Switzerland (Zurfluh et al., 2013); 33 strains from the intestines of freshwater fish (Abgottspon et al., 2014a); 26 isolates from different types of fresh vegetables (basil, beans, bitter cucumber cha-om, coriander, chilli, curry leaves and okra) imported to Switzerland from the Dominican Republic, India, Thailand and Vietnam (Zurfluh et al., 2015); isolates from fecal samples of chicken (n=6), pigs (n=3), lamb (n=1) and cattle (n=1) collected from healthy animals entering the slaughterhouses (Geser et al, 2012); 17 samples originating from a longitudinal sampling study at 3 different broiler chicken farms distributed throughout Switzerland (Zurfluh et al., 2014); strains obtained from poultry meat (n=13) (Abgottspon et al., 2014b); strains originating from fecal samples of healthy humans (n=34) or from fecal swabs of primary care patients (n=13) in Switzerland (Geser et al., 2012; Nüesch-Inderbinen et al., 2013a). Sources and identities of all strains, as well as isolation dates are indicated in Figure S1.

**2.2 Virulence factor genes**

DNA from *E. coli* isolates was extracted by a standard boiling procedure and all 207 isolates were screened by PCR for six markers of virulence associated with UPEC and eight marker genes for IPEC. The UPEC marker genes and the EAEC-specific gene *aggR* were amplified by conventional PCR using primers and conditions described previously for *traT*, *fyuA* and PAI (Johnson & Stell, 2000), *chuA* and *yfcv* (Spurbeck et al., 2012), *vat* (Ewers et al., 2005) and *aggR* (Boisen et al., 2012), respectively.

The IPEC virulence factors *eae* (EPEC), STh, STp and LT (ETEC), *stx1* and *stx2* (STEC) and *ipaH* (EIEC) were detected by real time multiplex PCR (Light Cycler) using QuantiFast Multiplex PCR Kit, (Qiagen, Hombrechtikon, Switzerland), and primers and cycling conditions according to the guidelines of the European Union Reference Laboratory for *E. coli* (EU Reference Laboratory for *E. coli*, 2013).

The aggregate VF score was defined as the number of unique UPEC-VF detected for each isolate, counting the PAI marker as one. Such molecular characteristics predict the extraintestinal virulence potential of an *E. coli* isolate *in vivo* (Johnson et al., 2006).

**2.3. Multilocus sequence typing**

For multilocus sequence typing of *E. coli* isolates, internal fragments of the seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA*, and *recA*) were amplified by PCR from DNA, as described by Wirth *et al*. (Wirth et al., 2006). Sequencing of the amplification products was performed by Microsynth (Balgach). Sequences were imported into the *E. coli* MLST database website (http://mlst.ucc.ie/mlst/dbs/Ecoli) to determine MLST types. Alleles and STs that had not been previously described were assigned new designations by the curators of the database.

**2.4. Phylogenetic classification**

DNA from *E. coli* isolates were subjected to triplex PCR targeting the *chuA* gene, the *yjaA* gene and an unspecified DNA fragment termed TspE4.C2, as described previously (Clermont et al., 2000). Isolates were classified as belonging to one of the four phylogenetic groups A, B1, B2 or D, whereby group A and B1 typically contain commensal *E. coli* strains while groups B2 and D consist of virulent extra-intestinal strains (Johnson et al., 2001). Multilocus sequence typing and phylogenetic classification were performed on isolates from the strain collection that had not yet been characterized to this regard. Thus, 50 of the 60 isolates from rivers and lakes (Zurfluh et al., 2013) and 29 of the 34 isolates from healthy humans (Geser et al., 2012) were additionally typed.

**2.5. Statistical analysis**

Comparisons of proportions of virulence genes and proportions of endemic clones within the sources were performed by Fisher's exact test in a series of individual pairwise comparisons using 2x2 tables where each characteristic was determined as present or absent. The significance criterion was set at $p < 0.05$. Calculations were performed using the VassarStats website for statistical computation (http://www.vassarstats.net).

## 3. Results and Discussion

### 3.1 Distribution of virulence genes throughout the sources

*E. coli* harboring uropathogenic virulence factors (UPEC) were detected throughout the sources (Figure S1).Overall, 82% of the isolates tested positive for one or more markers of uropathogenic virulence. Among the 207 *E. coli* isolates, the prevalence of individual VF genes ranged from 0% (*vat*, *stx1*, *stx2*, *ipaH*) to 55% (*TraT*, a lipoprotein involved in serum resistance) (Table 1). Among the sources, the distribution of *TraT* was distinguished by a significantly lower prevalence for isolates from healthy humans compared to all other sources, ($p = 0.0001$, OR 0.1945, CI 0.08-0.45). The genes *chuA* and *fyuA* occurred at significantly lower rates in the isolates from the livestock source ($p=0.00242$, OR 0.3452, CI 0.1336-0.8924 and $p < 0.0001$, OR 0.1014, CI 0.03-0.35, respectively). Further, PAI (pathogenicity island) was significantly associated with isolates from healthy humans ($p < 0.0001$, OR 8.9728, CI 308-21) and vegetables ($p=0.0008$, OR 0.0723, CI 0.009-0.54). Hence, the presence or absence of PAI may be an important characteristic of a putative zoonotic or environmental strain in terms of potential transmission to humans.

The overall median aggregate VF scores (and ranges) of the isolates were the following: from surface water VF 1 (0-5), from fish 2 (0-5), from vegetables 1 (0-5), livestock 1 (range 0-5), retail meat 1 (1-5), healthy humans 2 (0-5) and primary care patients 3 (0-5).

186 The highest prevalences of VF were observed in isolates from primary care patients.
187 Accordingly, the mean aggregate VF score was highest among this group. However, the large
188 range of aggregate VF scores show that each source is contaminated with highly virulent
189 pathogenic *E. coli.* With regard to *fyuA* and *chuA* which are associated with a large mean of
190 other VF genes and have been described as predictors of UPEC (Spurbeck et al., 2012), a total
191 of 72 (34.8%)isolates containing both these genes may be considered UPEC. These putative
192 UPEC isolates showed the following distribution throughout the sources: surface water, 20 of
193 60 isolates (33.3%); fish, 17 of 33 isolates (51.5%); vegetables, 5 of 26 isolates (19.2%);
194 livestock 3 of 28 isolates (10.7%); retail meat, 4 of 13 isolates (30.8%); healthy humans, 15 of
195 34 isolates (44.1%); and primary care patients, 8 of 13 isolates (61.5%)."
196 It is known from previous studies that uropathogenic *E. coli* can survive wastewater treatment
197 and are released into surface waters (Anastasi et al., 2010). Pollution of water and the
198 detection of uropathogenic isolates in freshwater fish destined for human consumption is of
199 major concern. Guzman and collaborators (Guzmán et at., 2004) have shown that there is
200 positive linear relationship between the concentration of *E. coli* in the digestive tract and the
201 edible tissue of freshwater fish. Consequently, freshwater fish from polluted surface waters
202 must be considered a potential risk factor for acquiring UPEC by handling or by cross-
203 contamination during preparation. Currently, much attention is focused on the contamination
204 of fresh produce with intestinal pathogens (Brandl & Sundin, 2013), but little is known about
205 the risk of acquiring UPEC from fruit and vegetables. The samples analyzed in this study
206 originated from India, Thailand, Vietnam or the Dominican Republic, where wastewater
207 without, or with insufficient treatment is commonly used for horticultural production (Zurfluh
208 et al., 2015). Our data show that 17 of 26 (65.4%) of the vegetable isolates harbored one or
209 more uropathogenic VF. Although the presence of a single VF gene is not sufficient to lable
210 an isolate UPEC, these results highlight the need to broaden the focus on food-borne UTI to
211 include food of non-animal origins.

212 Regarding virulence factors for intestinal pathogenic *E. coli*, these were predominantly
213 associated with the aquatic environment, with *aggR* (EAEC) found in isolates from surface
214 waters and STp and LT associated with isolates from fish (Table 1). "As expected, *aggR* was
215 not detected among isolates from healthy humans, since individuals with intestinal infections
216 were not included in this group. Although healthy humans may be carriers of EAEC, *aggR* is
217 significantly associated with diarrhoeagenic strains (Nüesch-Inderbinen et al., 2013b).
218
219 **3.2. Distribution of virulence genes among the phylogenetic groups**
220 In accordance to their phylogenetic characteristics, the aggregate VF scores and ranges were
221 low in group A (median 1, range 0-3) and group B1 (median 1, range 0-2) and followed an
222 ascending gradient from through group D (median 3, range 1-5) to group B2 (median 5, range
223 2-5). Although classically defined as commensal (Clermont et al., 2000), 67.7% and 71.7% of
224 the isolates of phylogenetic groups A and B1, respectively, harbored one or more VF marker
225 (Table 2). Of the group A isolates, 33.9% harbored *fyuA*, which is described as an excellent
226 predictor gene for a large number of other VF (Spurbeck et al., 2012). Furthermore, the
227 pathogenicity island PAI detected in 19.4%, and 16.6% of group A and B1, respectively,
228 includes two *pap* operons encoding P fimbriae and the *hly*CABD hemolysin gene cluster
229 which are characteristics of highly virulent pyelonephritic *E. coli* (Kao et al, 1997). With
230 regard to uropathogenicity, *E. coli* of the A and B1 group appear to be pathotypically
231 heterogeneous.
232
233 **3. 3. Distribution of clones and other sequence types among the sources.**
234 Phylogenetic groups within each source were stratified by clonal complex and VF scores were
235 assigned to major endemic clones. Clones were further stratified according to ESBL-types in
236 order to determine their prevalence within each source and to identify clones with host source
237 overlap. An overview is given in Table S1.

Clonal overlaps were detected for A:ST10(CTX-M-1), with prevalences markedly increased in the livestock source (chicken) and significantly associated with the healthy human source (p= 0.0299, OR 4.0887, CI 1.2-13.8). Previous studies identifying poultry meat as a possible zoonotic reservoir of UPEC are supported by the results of this study with regard to clone A:ST10 harboring CTX-M-1, which is a poultry-associated ESBL (Abgottspon et al., 2014b; Leverstein-van Hall et al., 2011; Zurfluh et al., 2014). This finding is a further strong indication, albeit no definite proof, of transmission from poultry to humans. By contrast, clone A:ST10(CTX-M-15), which included one isolate harboring the EAEC marker gene *aggR*, was significantly associated with water sources (p= 0.047, OR 4.3636, CI 1-19). Previously, multidrug resistant clone A:ST10 containing *aggR* and other EAEC marker genes has been implicated in an outbreak in Copenhagen (patients with UTIs), for which no common food source was identified (Olesen et al., 2012). Hence, the aquatic environment appears to be an important reservoir of at least some UTI-associated strains. Further IPEC-associated VFs STp (ETEC) and LT (ETEC), were found associated with isolates from freshwater fish (p=0.0248 and 0.0248, respectively, both with OR and CI = ∞).

Clone A:ST10(CTX-M-55) was detected exclusively in and significantly associated with, isolates originating from vegetables, all of which had been imported from Asia to Switzerland (Zurfluh et al., 2015). CTX-M-55 is an ESBL variant that is found increasingly in Asian regions (Xia et al., 2014). The emergence of these clones in imported food exemplifies the impact of global trade on putative UPEC reservoirs. Future monitoring of this specific clone may offer the possibility to pinpoint future transmission events, since so far it has not been detected in other sources in Switzerland.

Isolates belonging to phylogenetic group B1 were significantly associated with the livestock source (p=0.0001, OR 18.889, CI 7-51) and consisted in particular of B1:ST1056(CTX-M-1) isolates from healthy chicken and from chicken meat (Figure S1, Figure 1 and Table S1). One

sequence type belonging to B1:ST446(CTX-M-1) overlapped between livestock and healthy humans and was observed in one isolate each, respectively (Figure S1).

Multiple sources shared isolates belonging to B1:ST155, whereby B1:ST155(CTX-M-14) and B1:ST155(CTX-M-15) were restricted to the aquatic environment and vegetable sources, while B1:ST155(CTX-M-1) was detected in vegetable and livestock sources and was associated significantly with the chicken meat source (p=0.0474, OR 8.6364, CI 1.4-52).

The globally dominant pathogenic clone B2:ST131 was found most prevalently in water, in fish, and in primary care patients. This clone is mainly associated with hospital and community-acquired infections in humans (Rogers et al., 2011; Singer, 2015). In particular, B2:ST131(CTX-M-15) was not found in livestock, retail meat or healthy humans. This finding contrasts with previous studies that reported food-borne origins of this clone (Manges & Johnson, 2012). Likewise, B2:ST131(CTX-M-14) was not found in livestock or chicken meat, but, by contrast to B2:ST131(CTX-M-15), in healthy humans. B2:ST131(CTX-M-27) was isolated at a markedly increased rate from the aquatic environment (3 isolates/5%), including fish (p=0.0379, OR 4.6621, CI 1.2-18.4), and also from primary care patients (2 isolates/15.4%). This is supportive of previous studies that detected this clone in waterfowl and clinical settings (Micenková et al., 2014; Tausova et al., 2012; Zurfluh et al., 2013).

Clone B2:ST95 which is known to be shared between APEC and human ExPEC (Maluta et al., 2014) was detected in one healthy human.

Clones observed among phylogenetic group D included D:ST38, D:ST69, D:ST405 and D:ST648 (Table S1). They were detected at low rates in water, fish, vegetables and humans, but not in livestock or retail meat. *E. coli* D:ST648(CTX-M-15) has been described in companion animals and horses, and is proposed as a novel extraintestinal clone (Ewers et al., 2014). In this study it was found significantly associated with the healthy human source (p=0.015, OR 4.6621, CI 1.6-35.5). This may indicate its anthropogenic origin.

The only clone from group D shared by isolates from livestock (pig), chicken meat and healthy humans was D:ST350. This clone has been detected in APEC causing salpingitis and peritonitis and is also associated with UTI in humans (Pires-dos-Santos et al., 2013). Possibly, D:ST350 (CTX-M-1) represents a zoonotic genotype and food animals other than poultry, i.e. pork, may constitute a reservoir.

Clearly, future investigations should be undertaken to clarify whether ESBL-producing UPEC clones detected in healthy individuals are also associated with UTI in diseased humans.

## 4. Conclusions

Pathogenic *E. coli* is a major burden of disease worldwide. This study identifies potential reservoirs of pathogenic, ESBL-producing *E.coli* in the environment, animals, food and humans. *E. coli* harboring virulence factors that predict uropathogenicity were detected throughout the analyzed sources. Overall, 82% of the isolates tested positive for one or more virulence factors. Source overlaps between the aquatic environment, livestock, retail meat and healthy humans were noted for the clone A:ST10(CTX-M-1). Overlaps between the aquatic environment and healthy humans included clones B2:ST131(CTX-M-14), D:ST38(CTX-M-14), D:ST405(CTX-M-15) and D:ST648(CTX-M-15). Further, livestock, retail meat and healthy humans shared clone D:ST350(CTX-M-1).

The simultaneous analysis of different potential reservoirs of UPEC contributes to current understanding of the characteristics of multidrug resistant, uropathogenic *E. coli* at the interface between multiple sources.and may be useful for assessing potential risk factors for UPEC infection and for future studies aimed at determining the directionality of the dissemination of pathogenic *E. coli.*

## 5. Acknowledgements

314 The authors acknowledge Katrin Zurfluh for her technical support.

315

316 **6. Conflict of interest**

317 No conflict of interest declared.

318

## References


Abgottspon, H., Nüesch-Inderbinen, M. T., Zurfluh, K., Althaus, D., Hächler, H., Stephan, R. 2014a. Enterobacteriaceae with extended-spectrum-and pampc-type β-lactamase-encoding genes isolated from freshwater fish from two lakes in switzerland. Antimicro Agents Chemother 58(4), 2482-2484.

Abgottspon, H., Stephan, R., Bagutti, C., Brodmann, P., Hächler, H., Zurfluh, K. (2014b). Characteristics of extended-spectrum cephalosporin-resistant *Escherichia coli* isolated from Swiss and imported poultry meat. J Food Prot 77(1), 112-5. doi:10.4315/0362-028X.JFP-13-120.

Amos, G. C. A., Hawkey, P. M., Gaze, W. H., Wellington, E. M. (2014). Waste water effluent contributes to the dissemination of CTX-M-15 in the natural environment. J Antimicrob Chemother, dku079. doi:doi:10.1093/jac/dku079

Anastasi, E. M., Matthews, B., Gundogdu, A., Vollmerhausen, T. L., Ramos, N. L., Stratton, H., Katouli, M. (2010). Prevalence and persistence of *Escherichia coli* strains with uropathogenic virulence characteristics in sewage treatment plants. Appl Environ Microbiol 76(17), 5882-5886.

Boisen, N., Scheutz, F., Rasko, D. A., Redman, J. C., Persson, S., Simon, J., Kotloff KL, Levine MM, Sow S, Tamboura B, Toure A, Malle D, Panchalingam S, Krogfelt KA., Nataro, J. P. (2012). Genomic characterization of enteroaggregative *Escherichia coli* from children in Mali. J Inf Dis 205(3), 431-444. doi:10.1093/infdis/jir757.

Brandl, M. T., Sundin, G. W. (2013). Focus on food safety: Human pathogens on plants. Phytopathol 103(4), 304-305.

Clermont, O., Bonacorsi, S., Bingen, E. (2000). Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 66(10), 4555-4558.

343 Danzeisen, J. L., Wannemuehler, Y., Nolan, L. K., Johnson, T. J. (2013). Comparison of
344 multilocus sequence analysis and virulence genotyping of *Escherichia coli* from live birds,
345 retail poultry meat, and human extraintestinal infection. Avian Dis, 57(1), 104-8.
346 European Union Reference Laboratory for E. coli.(2006). Laboratory methods for VTEC
347 detection and typing. (Retrieved on June 21, 2015. Available at:
348 http://www.iss.it/vtec/index.php?lang=2&anno=2015&tipo=3).
349 Ewers, C., Bethe, A., Stamm, I., Grobbel, M., Kopp, P. A., Guerra, B., Stubbe M, Doi Y,
350 Zong Z, Kola A, Schaufler K, Semmler T, Fruth A, Wieler LH., Guenther, S. (2014).
351 CTX-M-15-D-ST648 *Escherichia coli* from companion animals and horses: Another clone
352 combining multiresistance and extraintestinal virulence? J Antimicrob Chemother, 69(5),
353 1224-30. doi:10.1093/jac/dkt516
354 Ewers, C., Janssen, T., Kiessling, S., Philipp, H. C., & Wieler, L. H. (2005). Rapid detection
355 of virulence-associated genes in avian pathogenic *Escherichia coli* by multiplex
356 polymerase chain reaction. Avian Dis, 49(2), 269-273.
357 Geser, N., Stephan, R., & Hächler, H. (2012). Occurrence and characteristics of extended-
358 spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals,
359 minced meat and raw milk. BMC Vet Res 8, 21. doi:10.1186/1746-6148-8-21
360 Geser, N., Stephan, R., Korczak, B. M., Beutin, L., & Hächler, H. (2012). Molecular
361 identification of extended-spectrum-β-lactamase genes from Enterobacteriaceae isolated
362 from healthy human carriers in Switzerland. Antimicrob Agents Chemother 56(3), 1609-
363 1612. doi:10.1128/AAC.05539-11.
364 Guzmán, M. C., Bistoni, M. d. e. . L., Tamagnini, L. M., & González, R. D. (2004). Recovery
365 of *Escherichia coli* in fresh water fish, Jenynsia multidentata and Bryconamericus iheringi.
366 Water Res, 38(9), 2367-73. doi:10.1016/j.watres.2004.02.016.

Johnson, J. R., & Stell, A. L. (2000). Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infs Dis 181(1), 261-272. doi:10.1086/315217.

Johnson, J. R., Clermont, O., Menard, M., Kuskowski, M. A., Picard, B., & Denamur, E. (2006). Experimental mouse lethality of *Escherichia coli* isolates, in relation to accessory traits, phylogenetic group, and ecological source. J Inf Dis 194(8), 1141-1150. doi:10.1086/507305

Johnson, J. R., Delavari, P., Kuskowski, M., & Stell, A. L. (2001). Phylogenetic distribution of extraintestinal virulence-associated traits in *Escherichia coli*. J Inf Dis 183(1), 78-88. doi:10.1086/317656

Johnson, J. R., Murray, A. C., Gajewski, A., Sullivan, M., Snippes, P., Kuskowski, M. A., & Smith, K. E. (2003). Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob Agents Chemother 47(7), 2161-2168. doi:10.1128/AAC.47.7.2161-2168.2003

Kao, J. S., Stucker, D. M., Warren, J. W., & Mobley, H. L. (1997). Pathogenicity island sequences of pyelonephritogenic *Escherichia coli* CFT073 are associated with virulent uropathogenic strains. Infect Immun 65(7), 2812-2820.

Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic *Escherichia coli*. Nature Rev Microbiol 2(2), 123-140. doi:10.1038/nrmicro818.

Leverstein-van Hall, M. A., Dierikx, C. M., Cohen Stuart, J., Voets, G. M., van den Munckhof, M. P., van Essen-Zandbergen, A., Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJ, Mevius DJ., National ESBL surveillance group. (2011). Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect 17(6), 873-880. doi:10.1111/j.1469-0691.2011.03497.x

Maluta, R. P., Logue, C. M., Casas, M. R., Meng, T., Guastalli, E. A., Rojas, T. C., Montelli AC, Sadatsune T, de Carvalho Ramos M, Nolan LK,. da Silveira, W. D. (2014).

393 Overlapped sequence types (sts) and serogroups of avian pathogenic (APEC) and human
394 extra-intestinal pathogenic (Expec) *Escherichia coli* isolated in Brazil. PloS One 9(8),
395 e105016. doi:10.1371/journal.pone.0105016.
396 Manges, A. R., & Johnson, J. R. (2012). Food-borne origins of *Escherichia coli* causing
397 extraintestinal infections. Clin Inf Dis 55(5), 712-719. doi:10.1093/cid/cis502.
398 Marrs, C. F., Zhang, L., Foxman, B. (2005). *Escherichia coli* mediated urinary tract
399 infections: Are there distinct uropathogenic *E. coli* (UPEC) pathotypes? FEMS Microbiol
400 Lett 252(2), 183-190. doi:10.1016/j.femsle.2005.08.028.
401 Micenková, L., Sisková, P., Bosák, J., Jamborová, I., Černohorská, L.,Smajs, D. (2014).
402 Characterization of human uropathogenic ESBL-producing *Escherichia coli* in the Czech
403 Republic: Spread of CTX-M-27-producing strains in a university hospital. Microb Drug
404 Res 20(6), 610-617.
405 Nataro, J. P., Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 11(1),
406 142-201.
407 Nüesch-Inderbinen, M. T., Abgottspon, H., Zurfluh, K., Nüesch, H. J., Stephan, R., Hächler,
408 H. (2013). Cross-Sectional study on fecal carriage of Enterobacteriaceae with resistance to
409 extended-spectrum cephalosporins in primary care patients. Microb Drug
410 Res.doi:10.1089/mdr.2013.0013.
411 Nüesch-Inderbinen MT, Hofer E, Hächler H, Beutin L, Stephan R. (2013). Characteristics of
412 enteroaggregative *Escherichia coli* isolated from healthy carriers and from patients with
413 diarrhoea. J Med Microbiol 62: 1828-1834.
414 Olesen, B., Scheutz, F., Andersen, R. L., Menard, M., Boisen, N., Johnston, B., Hansen DS,
415 Krogfelt KA, Nataro JP., Johnson, J. R. (2012). Enteroaggregative *Escherichia coli*
416 O78:H10, the cause of an outbreak of urinary tract infection. J Clin Microbiol, 50(11),
417 3703-11. doi:10.1128/JCM.01909-12

418 Pires-dos-Santos, T., Bisgaard, M., & Christensen, H. (2013). Genetic diversity and virulence
419 profiles of *Escherichia coli* causing salpingitis and peritonitis in broiler breeders. Vet
420 Microbiol 162(2-4), 873-880. doi:10.1016/j.vetmic.2012.11.008
421 Pitout, J. D. (2012). Extraintestinal pathogenic *Escherichia coli*: A combination of virulence
422 with antibiotic resistance. Fronts in Microbiol 3, 9. doi:10.3389/fmicb.2012.00009.
423 Rogers BA, Sidjabat HE, Paterson DL. *Escherichia coli* O25b-ST131: a pandemic,
424 multiresistant, community-associated strain. J Antimicrob Chemother 66: 1-14, 2011.
425 Singer, R. S. (2015). Urinary tract infections attributed to diverse ExPEC strains in food
426 animals: Evidence and data gaps. Fronts in Microbiol, 6, 28.
427 doi:10.3389/fmicb.2015.00028.
428 Spurbeck, R. R., Dinh, P. C., Walk, S. T., Stapleton, A. E., Hooton, T. M., Nolan, L. K., Kim
429 KS, Johnson JR., Mobley, H. L. (2012). *Escherichia coli* isolates that carry *vat*, *fyuA*, *chuA*,
430 and *yfcv* efficiently colonize the urinary tract. Infect Immun 80(12), 4115-22.
431 doi:10.1128/IAI.00752-12.
432 Tausova, D., Dolejska, M., Cizek, A., Hanusova, L., Hrusakova, J., Svoboda, O., Camlik G.,
433 Literak, I. (2012). *Escherichia coli* with extended-spectrum β-lactamase and plasmid-
434 mediated quinolone resistance genes in great cormorants and mallards in central Europe. J
435 Antimicrob Chemother 67(5), 1103-1107. doi:10.1093/jac/dks017
436 Totsika, M., Gomes Moriel, D., Idris, A., A Rogers, B., J Wurpel, D., Phan, M. -D., Paterson
437 D., A Schembri, M. (2012). Uropathogenic *Escherichia coli* mediated urinary tract
438 infection. Curr Drug Targets 13(11), 1386-1399.
439 Wiles, T. J., Kulesus, R. R., Mulvey, M. A. (2008). Origins and virulence mechanisms of
440 uropathogenic *Escherichia coli.* Exper Molr Pathol 85(1), 11-9.
441 doi:10.1016/j.yexmp.2008.03.007
442 Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L. H., Karch H, Reeves PR,
443 Maiden MC, Ochman H,. Achtman, M. (2006). Sex and virulence in *Escherichia coli:* An

444 evolutionary perspective. Mol Microbiol 60(5), 1136-1151. doi:10.1111/j.1365-
445 2958.2006.05172.x.
446 Xia S, Fan X, Huang Z, Xia L, Xiao M, Chen R, Xu Y, Zhuo C. (2014). Dominance of CTX-
447 M-type extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolated from
448 patients with community-onset and hospital-onset infection in China. 7. PLoS One
449 9:e100707
450 Zurfluh, K., Hächler, H., Nüesch-Inderbinen, M., Stephan, R. (2013). Characteristics of
451 extended-spectrum β-lactamase- and carbapenemase-producing Enterobacteriaceae isolates
452 from rivers and lakes in switzerland. Applied and Environmental Microbiology, 79(9),
453 3021-6. doi:10.1128/AEM.00054-13
454 Zurfluh, K., Jakobi, G., Stephan, R., Hächler, H., Nüesch-Inderbinen, M. (2014). Replicon
455 typing of plasmids carrying bla CTX-M-1 in Enterobacteriaceae of animal, environmental
456 and human origin. Front in Microbiol 5, 555. doi:10.3389/fmicb.2014.00555.
457 Zurfluh, K., Nüesch-Inderbinen, M., Morach, M., Berner, A. Z., Hächler, H., Stephan, R.
458 (2015). Extended-spectrum ß-lactamase-producing-Enterobacteriaceae in vegetables
459 imported from the Dominican Republic, India, Thailand and Vietnam. Appl Environ
460 Microbiol 81, 3115-3120. doi:10.1128/AEM.00258-15
461 Zurfluh, K., Nüesch-Inderbinen, M., Stephan, R., Hächler, H. (2013). Higher-generation
462 cephalosporin-resistant *Escherichia coli* in feral birds in Switzerland. Int J Antimicrob
463 Agents 41(3), 296-297.
464 Zurfluh, K., Wang, J., Klumpp, J., Nüesch-Inderbinen, M., Fanning, S., Stephan, R. (2014).
465 Vertical transmission of highly similar blaCTX-M-1-harboring Inci1 plasmids in
466 *Escherichia coli* with different MLST types in the poultry production pyramid. Fronts in
467 Microbiol 5, Article 519. doi: 10.3389/fmicb.2014.00519.
468
469